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Familial Neurofibromatosis 1 Microdeletions: Cosegregation With Distinct Facial Phenotype and Early Onset of Cutaneous Neurofibromata

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A notable subset of the recent literature on the disorder neurofibromatosis type 1 (NF1) describes patients with NF1, facial anomalies, and other unusual findings. We describe a molecular re-evaluation of two such families reported previously by Kaplan and Rosenblatt [1985], who suggested that their NF1 manifestations, facial phenotype, and other findings could result from a disorder distinct from NF1. Submicroscopic deletions involving the *NF1* gene were identified in both families by fluorescent in situ hybridization and analysis of somatic cell hybrids. Affected subjects of the first family were heterozygous for a microdeletion of approximately 2 Mb, which included the entire *NF1* gene and flanking contiguous sequences. The family was remarkable for cosegregation of the *NF1* microdeletion with facial abnormalities and a pattern of early onset of cutaneous neurofibromata upon transmission from an affected mother to her three affected children. The propositus of the second family carried a deletion that at the least involved *NF1* exon 2 through intron 27, which is ≥ 200 kilobases in length. Because all persons in the family were deceased, the size of the deletion could not be determined precisely. Facial anomalies were observed in the propositus and his NF1-affected mother and sister. The data from these families support our hypothesis, which was initially based solely on sporadic deletion cases, that deletion of the entire *NF1* gene, or in conjunction with deletion of unknown contiguous genes, causes the facial anomalies and early onset of neurofibromata observed in this subset of NF1 patients. In addition, other features observed in the persons in these families suggest that some *NF1* microdeletion patients may be at increased risk for connective tissue abnormalities and/or neoplasms. Am. J. Med. Genet. 73:197–204, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: neurofibromata; tumorigenesis; contiguous gene deletion

INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common autosomal disorder characterized primarily by multiple neurofibromata, café-au-lait spots, axillary/inguinal freckling, Lisch nodules, optic gliomas, and bony abnormalities [Riccardi, 1992; Viskochil and Carey, 1992]. Recently some 15 reports have described patients with physical manifestations of NF1 along with facial anomalies and other unusual findings. In some cases, the patient phenotype was sufficiently unusual that the diagnosis of a new undefined disorder was hypothesized [e.g., Kaplan and Rosenblatt, 1985; Allanson et al., 1985; Quattrin et al., 1987; Borochowitz et al., 1989; Opitz and Weaver, 1985; Abuelo and Meryash, 1988]. Explanations proposed for the unusual combination of findings in these patients included coincidental association, clinical variability of NF1, contiguous

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gene deletion, and a previously undefined genetic disorder [e.g., Abuelo and Meryash, 1988; Hall and Allanson, 1991; Stern et al., 1992; Buehning and Curry, 1995]. While careful clinical observations led to the recognition of these interesting atypical patients, molecular analyses will be necessary to resolve their diagnosis.

NF1 gene deletions have been identified in some NF1 patients with facial anomalies and unusual findings. In 1992, we reported on the first patient with a submicroscopic deletion spanning the *NF1* gene and have since extended that study to a total of seven cases [Kayes et al., 1992, 1994: Leppig et al., 1996]. The deletions were >700 kilobases in size and involved at least four known genes, *NF1* and the three genes embedded within an *NF1* intron, *EVI2A, EVI2B,* and *OMG.* These patients were remarkable for facial anomalies, early onset of cutaneous neurofibromata, no family history of NF1, and variable cognitive deficits ranging from mild learning disabilities to mental retardation. Because learning disabilities occur in approximately 30% of NF1 patients, any correlation between deletion and degree of cognitive impairment was not clear. These data led us to hypothesize that deletion of either the entire *NF1* gene or the *NF1* gene along with an unknown contiguous gene, predisposed to the development of the minor anomalies and early onset cutaneous neurofibromata (prior to age 10), and the speculation that such patients may be at increased risk for neoplasia [Kayes et al., 1994; Leppig et al., 1996]. Consistent with our hypothesis, other *NF1* gene deletion patients have been reported recently with similar phenotype [Wu et al., 1996, 1997; Colley et al., 1996; Ainsworth et al., 1997]. Not all patients with facial anomalies, however, carry submicroscopic deletions of the *NF1* gene [Tassabehji et al., 1993; Kayes et al., 1994; Wu et al., 1996].

Upon reviewing reports from the 1980s of NF1 patients with unusual facial appearance, we hypothesized that some of them could be carrying *NF1* microdeletions. In this report, we describe the molecular reevaluation of two unrelated NF1 families reported previously by Kaplan and Rosenblatt [1985]. At that time, their unusual facial and other findings suggested they may have a condition other than NF1. We have determined that both propositi carried submicroscopic deletions involving the *NF1* gene. This provided the opportunity to determine whether the deletion cosegregated with facial anomalies and early onset of cutaneous neurofibromata in family members.

MATERIALS AND METHODS

Subjects

For clarity of discussion, the original designations of families A and B [Kaplan and Rosenblatt, 1985] were changed to unique identifiers UWA169 and UWA166, respectively. The UWA166 family was reexamined in 1994. The propositus UWA166-2, age 16 years, had an occipital-frontal circumference (OFC) of 56.5 cm (85%), height of 170.5 cm (25%), and weight of 71.1 kg. His armspan was 179.0 cm, and he had an upper to lower body segment ratio of 0.97 (50%). He had malar flattening, a triangular facial appearance, and telecanthus (inner canthal distance 4.2 cm, outer canthal distance 9.1 cm, and interpupillary distance 6.0 cm). Patient UWA166-4, age 6 years, had an OFC of 48.5 cm (25%) and height of 113.7 cm (50%). Her right hand measured 13.5 cm (75%) with the right index finger measuring 6 cm (80%) and right foot 18.5 cm (75%). Patient UWA166-3, age 4 years, had an OFC of 49 cm (35%), height of 101 cm (50%), and weight of 15.25 kg. Telecanthus was present: inner canthal distance 3.4 cm, outer canthal 7.2 cm, and interpupillary 5 cm. His right hand measured 13 cm, right index finger 5.5 cm, and right foot 18 cm (all 97%). Numbers of neurofibromata at a given age were from the population-based study of Huson et al. [1988].

FISH Analyses

Chromosomes were prepared from Epstein-Barr virus transformed lymphoblasts from the mother and three children of family UWA166 and from fibroblasts from propositus UWA169-1 by using standard techniques that included exposure to 0.1 μg/ ml of colcemid, treatment with 0.075 KCl, and 3:1 methanol and acetic acid fixative. Fluorescence in situ hybridization (FISH) was performed as described previously with probe P1-9, which contains approximately 65 kb of the *NF1* gene including exons 2 through 11, and probe P1-12, which carries approximately 55 kb of the *NF1* gene including intron 27B [Leppig et al., 1996]. A minimum of 20 scorable metaphase cells, with both homologues of chromosome 17 clearly identifiable, were analyzed for each patient/probe combination. Symmetrical hybridization signals on both chromatids of a chromosome 17 was scored as an intact *NF1* locus, while absence of hybridization signals on both chromatids of a chromosome 17 was scored as an *NF1* deletion.

NF1 Deletion Mapping

DNA was purified from peripheral blood of members of family UW166 and from fibroblasts of UWA169-1, which had been banked prior to his death. Immortalized lymphoblastoid cell lines were constructed by Epstein-Barr virus transformation for individuals of family UW166 [Neitzel, 1986]. Individual chromosome 17 homologues were isolated from immortalized lymphoblastoid cell lines by constructing human/hamster somatic cell hybrid lines for patient UWA166-2 as described previously [Kayes et al., 1994]. The hybrid lines of UWA166-2 and genomic DNA from other family members were genotyped at 12 loci by polymerase chain reaction (PCR) amplification of polymorphic sites at D17S33 [Ainsworth and Rodenhiser, 1991], UT172 [Shannon et al., 1994], *NF1* exon 5 [Hoffmeyer and Assum, 1994], *NF1* intron 27B AluI/Alu II [Xu et al. 1991], *NF1* intron 38 [Lazaro et al., 1993], D17S57 and D17S73 [Rodenhiser et al., 1993], and D17S800 and D17S791 [Gyapay et al., 1994]. The presence or absence of the CRYB1 locus and AH1 and AN2, loci from the termini of a 700 kb *NF1* YAC contig [Marchuk et al., 1992], was determined in hybrid cell lines by PCR [Leach, 1991; Stephens, personal communication]. D17S800 and D17S791 loci were physically mapped telomeric to both *NF1* and D17S73 by their presence or

absence in three hybrid lines carrying partial chromosome 17s including NF13 [Ledbetter et al., 1989], SP3-10 [van Tuinen et al., 1987], and UWA106-3-hybrid 36 [Kayes et al., 1994].

RESULTS

Identification of *NF1* Gene Deletions

The phenotype of the propositi described previously by Kaplan and Rosenblatt [1985] made them and their affected relatives likely candidates for carriers of deletions encompassing *NF1*. FISH of clone P1-9, which harbors *NF1* exons 2–11, to chromosomes of propositus UWA166-2 demonstrated that only one homologue of chromosome 17 showed a positive hybridization signal in each of 20 metaphase cells examined (Fig. 1A). Hybridization of chromosome preparations prepared from affected family members, including mother, half-sister, and half-brother (Fig. 2), with clone P1-9 demonstrated that they also carried a hetererozygous deletion of the *NF1* gene (data not shown). To delineate the extent of the deletion, two human/hamster somatic cell hybrid lines were constructed each of which carried one of the human chromosomes 17 from the propositus UWA166-2. The haplotypes of the *NF1* region were determined unambiguously for UWA166-2 by genotyping 12 loci in each somatic cell hybrid line (Fig. 2). While the paternal chromosome 17 was intact, the maternal chromosome carried a deletion that spanned the entire 350 kb *NF1* gene and large regions of flanking DNA. The proximal deletion breakpoint was mapped between CRYB1 and UT172 and the distal breakpoint between AN2 and D17S57. As expected, the haplotypes of other relatives demonstrated that all three affected children inherited the deleted chromosome 17 from their affected mother (Fig. 2).

Chromosomes from the second propositus, UWA169-1, were hybridized independently to two different probes from the *NF1* locus. Both probes P1-9 and P1-12 hybridized to only one homologue of chromosome 17 in each of 20 metaphase cells examined (Fig. 1B). These data demonstrated that patient UWA169-1 carried a deletion of one *NF1* allele that minimally involved exons 2 through intron 27B. Although lack of somatic cell hybrids or parental DNA precluded detailed mapping of this deletion, cytogenetic observation of the centromere and heterozygosity at D17S73 defined the maximum limits of the deletion.

Patient Phenotypes

At the time of the initial description of these families, patient UWA166-2 was a 7-year-old boy with multiple café-au-lait spots, several cutaneous neurofibromata, speckling of the iris, and distinctive facial appearance [Kaplan and Rosenblatt, 1985]. His findings at 16 years are summarized in Table I. The number of tumors had increased to 19 cutaneous neurofibromata and one small apparent plexiform neurofibroma on his back. In addition, he had a patch of pebbly-textured skin on his upper back, an anomaly also found in other *NF1* deletion patients and presumed to be numerous nascent neurofibromata [Kayes et al., 1994]. In addition, the patient gave a history of possible dislocation of the radial heads of both arms. He refused to have his photo-

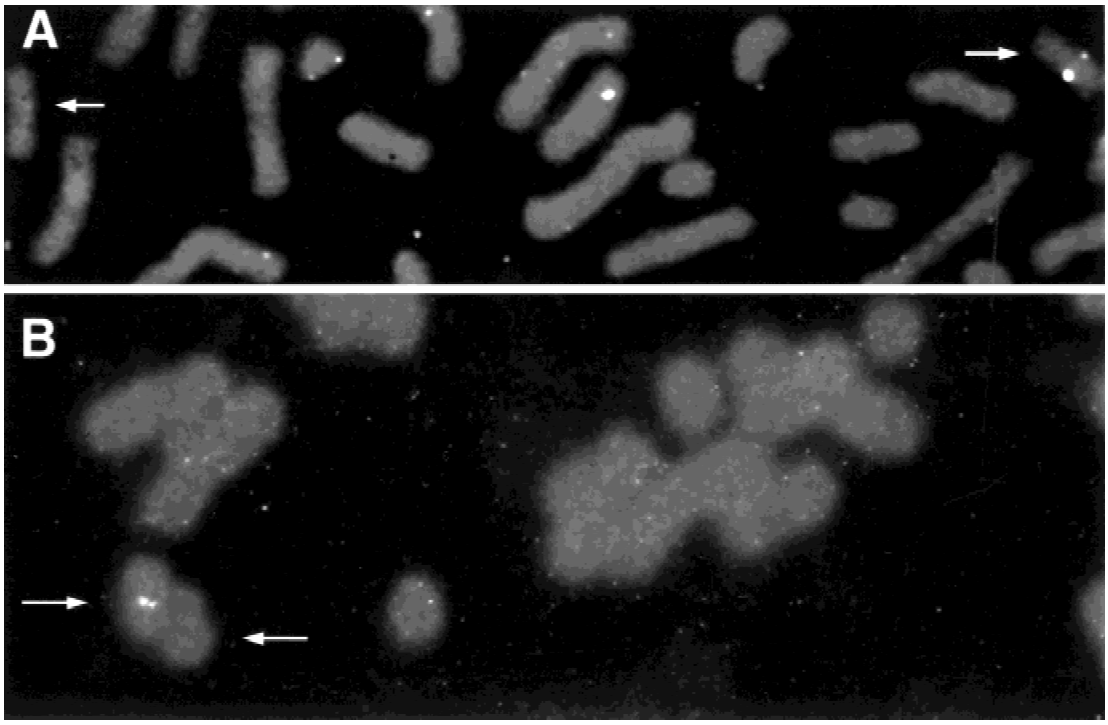

Fig. 1. Detection of *NF1* gene deletion by FISH. **A:** Hybridization of probe P1-9 to metaphase cells from UWA166-2 showing signal on one homologue only. **B:** Hybridization of clone P1-12 to metaphase cells from UWA169-1 showing signal on one chromosome 17 homologue only. Chromosome 17 homologues were identified by the Hoechst/actinomycin D staining (data not shown), which reveals a Q-banding like pattern [Chance et al., 1993].

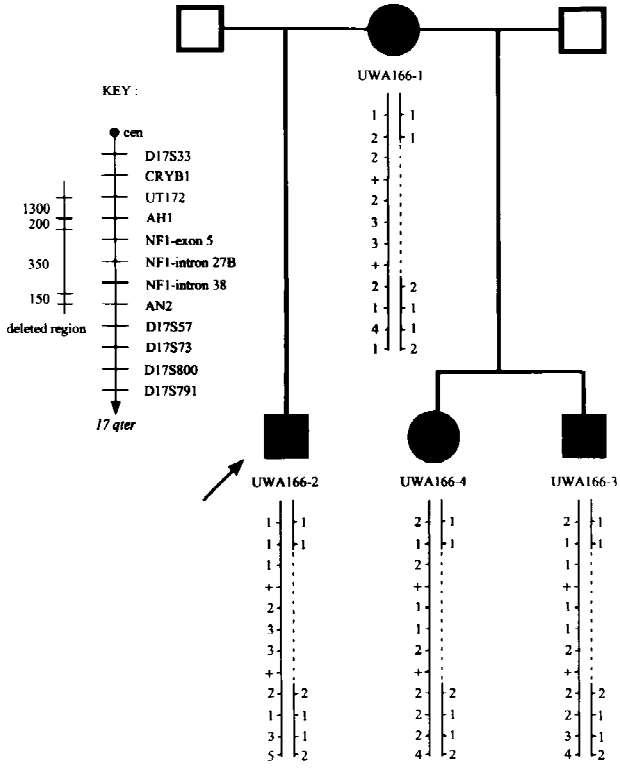

Fig. 2. Pedigree and haplotypes of family UWA166. Haplotypes of propositus UWA166-2 were determined by genotypic analyses of 12 loci in each chromosome 17 isolated in two human/hamster somatic cell hybrid lines. These data, in addition to marker alleles and presence of *NF1* deletion as determined by FISH, were used to deduce haplotypes of other relatives. Schematic of unique loci order is not drawn to scale; physical map of deleted region is given in kilobase pairs [Marchuk et al., 1992; Shannon et al., 1994].

graph taken during this evaluation. His mother, designated as patient UWA166-1, was interviewed but not re-examined fully since the original description of her findings [Kaplan and Rosenblatt, 1985]. She now had two additional children, fathered by a different partner, who were evaluated fully. As documented in Table I and Figures 2 and 3, each affected relative carried an *NF1* microdeletion and had remarkably similar facial findings.

Figure 3 shows the "coarse" facial appearance of UWA169-1 at age 17 [Kaplan and Rosenblatt, 1985], including triangular shape of face, down-slanting palpebral fisssures with slight eversion of the lower eyelid, telecanthus, broad high nasal root, and a wide appearing neck as the result of a high insertion of the trapezius. Patient UWA169-1 died of complications during aortic valve replacement surgery in his second decade. An echocardiogram at age 12 showed a dilated aortic root of 40 mm with aortic incompetence and mitral valve prolapse. The aortic valve, which was not bicuspid, was replaced with a prosthetic valve at age 16; death occurred during subsequent valve replacement. Examination of an old family photograph of his deceased mother, who also had NF1, demonstrates apparent telecanthus, down-slanting palpebral fissures, large nose, high nasal bridge, broad neck, and marfanoid habitus (dolichostenomelia) (Fig. 3).

DISCUSSION

We have identified a family with multiple individuals affected with NF1 by virtue of inheriting a submicroscopic deletion spanning the *NF1* gene. FISH and somatic cell hybrid analysis demonstrated that all three affected children in family UWA166 inherited the *NF1*-deleted chromosome 17 from their affected mother. Each affected relative was hemizygous for four known genes, *NF1* and *OMG, EVI2A,* and *EVI2B,* the three genes encoded by the complimentary strand of the *NF1* gene [reviewed in Viskochil et al., 1993]. The deletion, bounded by the β-crystallin gene (CRYB1) and D17S57, was estimated to be 2 Mb in length (Fig. 2). This deletion is comparable in size and extent to deletions mapped in other patients, which were originally estimated at >700 kb [Kayes et al., 1994] and subsequently refined to approximately 2 Mb due to the inclusion of the UT172 locus estimated at 1.5 Mb centromeric of *NF1* [Stephens, personal communication; Shannon et al., 1994].

The propositus of a second family, UWA169-1, also carried an *NF1* gene deletion, which at the least included exon 2 through intron 27B of the *NF1* gene or a minimum of 200 kb (≥60%) of the *NF1* gene [Li et al., 1995]. It is not known if the deletion in this patient extended into contiguous DNA sequences. Attempts to map the deletion were thwarted by failure of fibroblasts of the deceased propositus to fuse with rodent cells for construction of hybrid cell lines and equivocal results with probes flanking the *NF1* gene in quantitative Southern blot analyses. In addition, lack of tissues from both the deceased affected mother and sister precluded molecular confirmation of a deletion in these individuals. However, their presentation of multiple café-au-lait spots, multiple neurofibromata, Lisch nodules, and malignant tumors were sufficient to confirm a diagnosis of NF1 [Kaplan and Rosenblatt, 1985], presumably caused by the same deletion carried by the propositus UWA169-1.

The *NF1* microdeletion cosegregated with an abnormal facial phenotype. This is demonstrated clearly in family UWA166 by comparison of features in Table I and photographs in Figure 3. The facial appearance of 4-year-old UWA166-3 was remarkably similar to that of his half-brother, UWA166-2, at age 7 (Fig. 3B,D). The facial changes of the three affected individuals in family UWA169 are documented by evaluation of the propositus UWA169-1, his affected mother (Fig. 3E,F), and affected sister [see Kaplan and Rosenblatt, 1985]. The resemblance among the individuals in these two families was remarkable, despite differences in age and ethnic heritage of French Canadian and Ashkenazi Jewish, respectively. Indeed, the facial appearance of UWA166-2 had coarsened in the intervening years since the previous evaluation and photograph at age 7, and as a teenager, he now bore a remarkable resemblance to UWA169-1 (Fig. 3).

The phenotype of subjects in family UWA166 support our earlier hypothesis that deletions spanning the *NF1* gene predispose to early onset of cutaneous neurofibromata at less than 10 years of age and/or numbers of neurofibromata >85th centile at a given age

TABLE I. Findings in Affected Relatives of Family UWA166

Findings/patients	UWA166-1	UWA166-2	UWA166-4	UWA166-3
Age last evaluation (years)	39[a]	16	6	4
Skin	Multiple CLS	Multiple CLS Freckling: axillary, inguinal, abdomen Soft redundant skin Excessive, wrinkled skin on both palms	Multiple CLS Freckling: axillary, abdomen Soft skin, increased elasticity Fleshy soles of feet Hairy nevus on knee [2 cm]	Multiple CLS Freckling: axillary, abdomen Soft skin Soft fleshy soles of feet
Neurofibromata	>100 cutaneous	19 cutaneous, 1 plexiform, many small nascent?	9 cutaneous	1 possible cutaneous
Skeletal	Scoliosis	Brachycephalic Overall joint laxity Mild pectus excavatum	Mildly brachycephalic Overall joint laxity Mild pectus excavatum	Overall joint laxity
	Large hands	Large hands and feet >97 centile	Large hands and feet >75 percentile	Large hands and feet >97 centile
Facial appearance	Telecanthus Down-slanting palpebral Fissures	Telecanthus Down-slanting palpebral fissures Eversion lower lateral eyelid[b]	Telecanthus Down-slanting palpebral fissures	Telecanthus Down-slanting palpebral fissures
	Eversion lower lateral eyelid	Large nose, high broad nasal bridge	Eversion lower lateral eyelid	
	Large nose, high nasal bridge	Normal set ears, thick helices	Large nose, high nasal bridge	High nasal bridge
	Normal set ears, thick helices	Small/pointed chin		Normal set ears, thick helices
	Small/pointed chin	Palate high narrow arch; teeth maloccluded	Small/pointed chin	Small/pointed chin
	Palate normal		Palate normal	Palate normal
	Broad neck	Broad neck, low hairline	Broad neck, normal hairline	Broad neck, low hairline
Other	Mild nonprogressive facial muscle weakness, dysphonia	Lisch nodules Gynecomastia, bilateral		Shawl scrotum
Cognitive/developmental delays	Speech impediment Dull intellect	Special education classes, 9th grade		Speech impediment
Previous evaluation	Kaplan and Rosenblatt, 1985	Kaplan and Rosenblatt, 1985	None	None

[a]Not fully re-evaluated at age 39.
[b]Everted lower lid, present at 7 years of age, absent at 16 years.

[Kayes et al., 1994; Leppig et al., 1996]. The three children in family UWA166 had early onset of cutaneous neurofibromata. At the age of 7, subject UWA166-2 had "several" neurofibromata and at 16 years, he had 19 cutaneous neurofibroma (90th centile). Subject UWA166-3 had 9 neurofibromata at age 6 (90th centile) and UWA166-4 had a possible neurofibroma at 4 years. For the deceased affected subjects of family UWA169, age of onset and tumor numbers are unknown. The association of early onset of cutaneous neurofibromata and facial anomalies with large NF1 deletions has been confirmed by others [Wu et al., 1996; 1997], although in these cases it is unknown whether the deletion was limited to *NF1* or also involved contiguous genes and sequences.

The molecular basis of early onset and increased numbers of neurofibromata is unclear. It seems unlikely that *NF1* haplo-insufficiency alone accounts for the phenotype. About 70% of *NF1* patients have mutations that predict premature truncation of neurofibromin [Heim et al., 1995] and yet the appearance of neurofibromata before age 10 was observed in less than 15% of patients [Huson et al., 1988]. Mutational inactivation of both *NF1* alleles has been documented in neurofibromata. Inactivating germline and somatic mutations were identified in both *NF1* alleles in a dermal neurofibroma from an NF1 patient [Sawada et al., 1996] and in the normal *NF1* allele of neurofibromata of patients who inherited an undefined germline *NF1* mutant allele [Colman et al., 1995]. This is consistent with a known function of neurofibromin as a negative regulator of the proto-oncogene *ras;* loss of neurofibromin activates the ras pathway and alters the normal cellular growth control in at least some cell types [Basu et al., 1992; DeClue et al., 1992; Bollag et al., 1996]. An interesting hypothesis is that germline deletion of either the *NF1* gene alone, or in conjunction with an unknown contiguous gene, predisposes to inactivation of the second *NF1* allele in a target cell, which eventually gives rise to a neurofibroma. In this hypothesis, the critical predisposing gene (or functional domain) would function normally to maintain the stability of

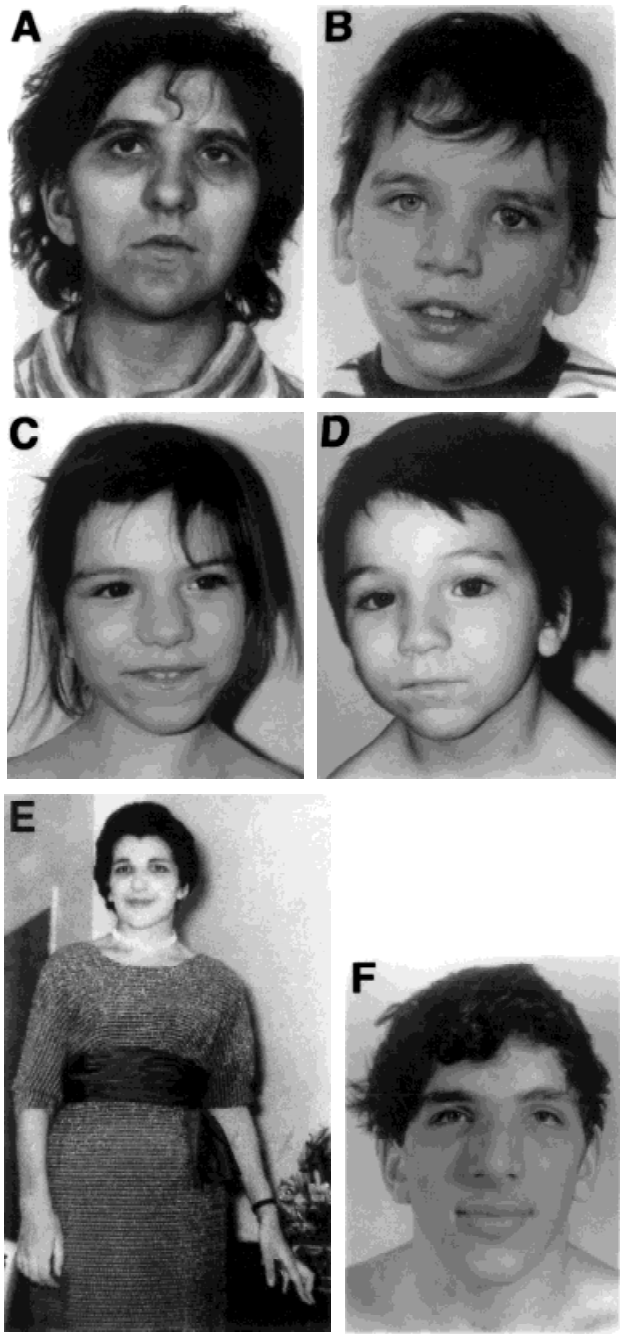

Fig. 3. Photographs of subjects with *NF1* microdeletions. **A:** Affected mother UWA166-1 at approximately 30 years of age. **B:** Affected son and propositus UWA166-2 at age 7 years. **C:** UWA166-4, maternal half sibling of propositus at 6 years. **D:** UWA166-3, maternal half-sibling of propositus at age 4 years. **E:** Deceased mother of propositus UWA169-1 at approximately 30 years. **F:** Propositus UWA169-1 at age 17 years. (A and B reprinted from Kaplan and Rosenblatt, 1985 with permission.)

the genome. Germline deletion of this putative "caretaker gene" [Kinzler and Vogelstein 1997] would increase the mutation rate, eventually leading to inactivation of the second *NF1* allele and possibly other critical loci, and tumorigenesis. Alternatively, the functional consequences of a 2 Mb germline *NF1* microdeletion could be to promote the outgrowth of specific target cells with preexisting somatic mutations in the remaining *NF1* allele. This epigenetic mechanism would thereby increase the phenotypic penetrance of somatic *NF1* mutations. This is a particularly appealing hypothesis if, as generally thought, the Schwann cell is the target cell for neurofibroma development. Division of Schwann cells is strictly regulated during fetal development but occurs at very low levels in normal adult nerves; second hit NF1 mutations in the fetus would occur in actively mitotic Schwann cells versus growth arrested Schwann cells in adults. Perhaps haplo-insufficiency for a critical protein in Schwann cells carrying germline NF1 microdeletions promotes proliferation of that subset of adult Schwann cells lacking neurofibromin. There is a precedence for such a mechanism of tumorigenesis in *N*-Nitroso-*N*-methylurea-induced rat mammary tumors. Zarbl and colleagues elegantly demonstrated that these tumors arise from cells with preexisting *Hras1* mutations, presumably due to mutagen-induced deregulation of *Hras1* expression [Cha et al., 1994; Jin et al., 1996].

It is unclear whether patients with *NF1* microdeletions are at increased risk of developing neoplasms. Homozygous inactivation of the *NF1* gene in a number of epidemiologically-associated neoplasms [Mulvihill 1994], including malignant myeloid cells, malignant peripheral nerve sheath tumors, and pheochromocytomas, strongly supports a tumor suppressor function for neurofibromin cells [Shannon et al., 1994; Miles et al., 1996; Legius et al., 1993; Xu et al., 1992]. Interestingly, three *NF1* microdeletion patients have died at a young age of tumors that are not known to be epidemiologically-associated with the *NF1* disorder, including cerebellar medulloblastoma, retroperitoneal fibrosarcoma, and cerebellar neuroblastoma [Materials and Methods; Kaplan and Rosenblatt, 1985; Wu et al., 1996]. Follow-up of NF1 microdeletion patients will determine whether this rearrangement predisposes to tumors not previously detected as being associated with the NF1 disorder.

In this study, affected members of family UWA166, and the propositus UW169-1, were found to have findings suggestive of a connective tissue disorder. As detailed in Table I, UWA166 subjects had joint laxity, an unusual redundance of skin on the palms and soles, and large hands and feet (Table I; Materials and Methods). UWA166-2 and UWA166-3 had a mild pectus excavatum. The propositus UWA169-1 had disproportionate tall stature, mild scoliosis, pectus excavatum, mild joint laxity, large hands and feet, and aortic dilatation in the second decade. His death during surgery to place a second prosthetic aortic valve resulted from friable aortic tissue which lacked strength and integrity. Detailed information and measurements were not available on his mother and sister, but his mother had a similar body habitus (Fig. 3).

Large hands and feet were also noted in retrospective review of medical records of other patients with *NF1* microdeletions [Kayes et al., 1994], and in two of four patients hemizygous for most, if not all of *NF1* [Wu et al., 1996]. In addition, one patient with a submicroscopic *NF1*-spanning deletion had Madelung deformity, a dominant trait marked by dorsal dislocation of the ulnar head [Leppig et al., 1966]. Together, these data

suggest the existence of an *NF1* contiguous gene involved in normal connective tissue development which was deleted or disrupted in these patients.

Although the data set is small, there appears to be a common phenotype associated with *NF1* contiguous gene deletions that includes minor facial anomalies and onset of cutaneous neurofibromata < age 10 years. Other anomalies observed in some of the subjects need to be examined in a larger data set to determine if they correlate with specific deleted regions. These anomalies include Madelung deformity [Leppig et al., 1996], skin and skeletal changes consistent with a connective tissue disorder [this article; Kayes et al., 1994; Wu et al., 1996], and neoplasms at a young age [this report; Wu et al., 1996].

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REFERENCES

Abuelo DN, Meryash DL (1988): Neurofibromatosis with fully expressed Noonan syndrome. Am J Med Genet 29:937–941.

Ainsworth PJ, Chakraborty PK, Weksberg R (1997): Example of somatic mosaicism in a series of de novo neurofibromatosis type 1 cases due to a maternally derived deletion. Hum Mutat 9:452–457.

Ainsworth PJ, Rodenhiser DI (1991): Rapid nonradioactive detection by PCR of pHHH202/RsaI RFLP linked to neurofibromatosis type 1. Am J Hum Genet 49:1098–1099.

Allanson JE, Hall JG, Van Allen MI (1985): Noonan phenotype associated with neurofibromatosis. Am J Med Genet 21:457–462.

Basu TN, Butmann DH, Fletcher JA, Glover TW, Collins FS, Downward J (1992): Aberrant regulation of *ras* proteins in malignant tumour cells from type 1 neurofibromatosis patients. Nature 356:713–715.

Bollag G, Clapp DW, Shih S, Adler F, Zhang Y, Thompson P, Lange BJ, Freedman M, McCormick F, Jacks T, Shannon K (1996): Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. Nat Genet 12:144–148.

Borochowitz Z, Berant N, Dar H, Berant M (1989): The neurofibromatosis-Noonan syndrome: genetic heterogeneity versus clinical variability. Neurofibromatosis 2:309–314.

Buehning L, Curry CJ (1995): Neurofibromatosis-Noonan syndrome. Ped Dermatol 12:267–271.

Cha RS, Thilly WG, Zarbl H (1994): *N*-Nitroso-*N*-methylureaa-induced rat mammary tumors arise from cells with preexisting oncogenic *Hras1* gene mutations. Proc Natl Acad Sci USA 91:3749–3753.

Chance PF, Anderson MK, Leppig KA, Lensch MW, Matsunami N, Smithe B, Swanson PD, Odelberg SJ, Disteche CM, Bird TD (1993): DNA deletions associated with hereditary liability to pressure palsies. Cell 72:143–151.

Colley A, Donnai D, Evans DGR (1996): Neurofibromatosis/Noonan phenotype: A variable feature of type 1 neurofibromatosis. Clin Genet 49: 59–64.

Colman SD, Williams CA, Wallace MR (1995): Benign neurofibromas in type 1 neurofibromatosis (NF1) shows somatic deletions of the NF1 gene. Nat Genet 11:90–92.

DeClue JE, Papageorge AG, Fletcher JA, Diehl SR, Ratner N, Vass WC, Lowy DR (1992): Abnormal regulation of mammalian $p21^{ras}$ contributes to malignant tumor growth in von Recklinghausen (Type 1) neurofibromatosis. Cell 69:265–273.

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Merc S, Bernardi G, Lathrop M, Weissenbach J (1994): The 1993–94 Genethon human genetic linkage map. Nat Genet 7:246–339.

Hall JG, Allanson JE (1991): Neurofibromatosis. I. Predicting the relation of gene structure to gene function. Am J Med Genet 38:135.

Heim RA, Kam-Morgan LNW, Binnie CG, Corns DD, Cayouette MC, Farber RA, Aylsworth AS, Silverman LM, Luce MC (1995) Distribution of 13 truncating mutations in the neurofibromatosis 1 gene. Hum Mol Genet 4:975–981.

Hoffmeyer S, Assum G (1994): An RsaI polymorphism in the transcribed region of the neurofibromatosis (*NF1*)-gene. Hum Genet 93:481–482.

Huson SM, Harper PS, Compston DAS (1988): Von Recklinghasen neurofibromatosis: A clinical and population study in south-east Wales. Brain 111:1355–1381.

Jin Z, Houle B, Mikheev AM, Cha RS, Zarbl H (1996): Alerations in *H-ras1* promoter conformation during *N*-Nitroso-*N*-methylureaa-induced mammary carcinogenesis and pregnancy. Cancer Res 56:4927–4935.

Kaplan P, Rosenblatt B (1985): A distinctive facial appearance in neurofibromatosis von Recklinghausen. Am J Med Genet 21:462–470.

Kayes LM, Burke W, Riccardi VM, Bennett RL, Ehrlich P, Rubenstein A, Stephens K (1994): Deletions spanning the neurofibromatosis 1 gene: Identification of the phenotype in five patients. Am J Hum Genet 54: 426–436.

Kayes LM, Riccardi VM, Burke W, Bennett RL, Stephens K (1992): Large de novo DNA deletion in a patient with sporadic neurofibromatosis, mental retardation and dysmorphism. J Med Genet 29:686–690.

Kinzler KW, Vogelstein B (1997): Cancer-susceptibility genes: Gatekeepers and caretakers. Nature 386:761–762.

Lazaro C, Gaona A, Xu G, Weiss R, Estivill X (1993): A highly informative Ca/GT repeat polymorphism in intron 38 of the human neurofibromatosis type 1 (*NF1*) gene. Hum Genet 92:429–430.

Leach RJ. Amplimers for CRYB1. 19 Mar 91. Personal communication in: GDB(Human Genome Database [database online]. Baltimore (MD): Johns Hopkins University, 1990–. Updated daily. Available from Internet: URL http://gdbwww.gdb.org/ [cited 5 June 1997; GDB node: USA, http://gdbwww.gdb.org/] Accession ID: CIT: 25472.

Ledbetter DH, Rich DC, O'Connell P, Leppert M, Carey JC (1989): Precise localization of *NF1* to 17q11.2 by balanced translocation. Am J Hum Genet 44:20–24.

Legius E, Marchuk DA, Collins FS, Glover TW (1993): Somatic deletion of the neurofibromatosis type 1 gene in a neurofibrosarcoma supports a tumour suppressor gene hypothesis. Nat Genet 3:122–126.

Leppig KA, Viskochil D, Neil S, Rubenstein A, Johnson VP, Zhu XL, Brothman AR, Stephens K (1996): The detection of contiguous gene deletions at the neurofibromatosis 1 locus with fluorescence in situ hybridization. Cygenet Cell Genet 72:95–98.

Li Y, O'Connell P, Breidenbach HH, Cawthon R, Stevens J, Xu G, Neil S, Robertson M White R, Viskochil D (1995): Genomic organization of the neurofibromatosis 1 gene. Genomics 25:9–18.

Marchuk DA, Tavakkol R, Wallace MR, Brownstein BH, Taillon-Miller P, Fong C-T, Legius E, Andersen LB, Glover TW, Collins FS (1992): A yeast artificial chromosome contig encompassing the type 1 neurofibromatosis gene. Genomics 13:672–680.

Miles DK, Freedman MH, Stephens K, Pallavicini M, Sievers EL, Weaver M, Grunberger T, Thompson P, Shannon KM (1996): Patterns of hematopoietic lineage involvement in children with neurofibromatosis type 1 and malignant myeloid disorders. Blood 88:4314–4320.

Mulvihill JJ (1984): Malignancy: epidemiologically associated cancers. In Huson SM, Hughes RAC (eds): "The Neurofibromatoses: A Pathogenetic and Clinical Overview." London: Chapman and Hall Medical, pp 305–315.

Neitzel H (1986): A routine method for the establishment of permanent growing lymphoblastoid cell lines. Hum Genet 73:320–326.

Opitz JM, Weaver DD (1985): The neurofibromatosis-Noonan syndrome. Am J Med Genet 21:477–490.

Quattrin T, McPherson E, Putnam T (1987): Vertical transmission of the neurofibromatosis/Noonan syndrome. Am J Med Genet 26:645–649.

Riccardi VM (1992): "Neurofibromatosis, Phenotype, Natural History, Pathogenesis." Baltimore: The Johns Hopkins University Press.

Rodenhiser DI, Ainsworth PJ, Coulter-Mackie MB, Singh SM, Jung JH (1993): A genetic study of neurofibromatosis type 1 (NF1) in southwestern Ontario. II. A PCR based approach to molecular and prenatal diagnosis using linkage. J Med Genet 30:363–368.

Sawada S, Florell S, Purandare SM, Ota M, Stephens K, Viskochil D

(1996): Identification of *NF1* mutations in both alleles of a dermal neurofibroma. Nat Genet 14:110–112.

Shannon KM, O'Connell P, Martin GA, Paderanga D, Olson K, Dinndorf P, McCormick F (1994): Loss of the normal *NF1* allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. N Engl J Med 330:597–601.

Stern HJ, Saal HM, Lee JS, Fain PR, Goldgar DE, Rosenbaum KN, Barker DF (1992): Clinical variability of type 1 neurofilbromatosis: Is there a neurofibromatosis-Noonan syndrome? J Med Genet 29:184–187.

Tassabehji M, Strachan T, Sharland M, Colley A, Donnai I, Harris R, Thakker N (1993): Tandem duplication within a neurofibromatosis type 1 (*NF1*) gene exon in a family with features of Watson syndrome and Noonan syndrome. Am J Hum Genet 53:90–95.

Van Tuinen P, Rich DC, Summers KM, Ledbetter DH (1987): Regional mapping panel for human chromosome 17: Application to neurofibromatosis type 1. Genomics 1:374–381.

Viskochil D, Carey JC (1992): Nosological considerations of the neurofibromatoses. J Dermatol 19:873–880.

Viskochil D, White R, Cawthon R (1993): The neurofibromatosis type 1 gene. Annu Rev Neurosci 16:183–205.

Wu B-L, Austin MA, Schneider GH, Boles RG, Korf BR (1996): Deletion of the entire *NF1* gene detected by FISH: four deletion patients associated with severe manifestations. Am J Med Genet 59:528–535.

Wu B-L, Schneider GH, Korf BR (1997): Deletion of the entire *NF1* gene causing distinct manifestations in a family. Am J Med Genet 69:98–101.

Xu G, Nelson L, O'Connell P, White R (1991): An alu polymorphism intragenic to the neurofibromatosis 1 gene (*NF1*). Nucleic Acids Res 19: 3764.

Xu W, Mulligan LM, Ponder MA, Liu L, Smith BA, Mathew CGP, Ponder BAJ (1992): Loss of *NF1* alleles in phaeochromocytomas from patients with type 1 neurofibromatosis. Genes Chrom Cancer 4:337–342.